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MICROTUBULE TARGETING DRUGS CAN THESE BE THE ANSWER TO THE SEARCH FOR AN ULTIMATE CANCER MEDICINE, A REVIEW

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ABSTRACT

Drugs acting on microtubules such as vinca alkaloids and taxanes have been used from a long time as chemotherapeutic agents for the treatment of cancer. These drugs bind to various binding sites on microtubules and show their effects either on microtubule dynamics or on microtubule polymer mass depending upon their mechanism of action and various factors like concentration of drug used. By binding to microtubules these drugs arrest cell cycle progression at metaphase of mitosis and eventually lead to apoptotic cell death. But the potential of these drugs to cure cancer could not be exploited completely because of various limiting factors such as side effects, of which the most notable ones are hematological and neurological toxicities. Drug resistance is another limiting factor which limits the effectiveness of these drugs. Efforts are on to overcome the limiting factors by various approaches like enclosing the drugs inside a carrier like liposome, by using latest drug delivery systems like nanoparticles and by using synergistic combinations of drugs. Research is also going on for the development and discovery of analogues with lesser side effects. At the same time researchers are also trying to get the drugs like colchicine into clinical use by finding ways to overcome its toxic effects because of which it could not be used previously.

KEY WORDS: Microtubules, Mitosis, Spindle Fibers, Apoptosis, Synergism.

INTRODUCTION

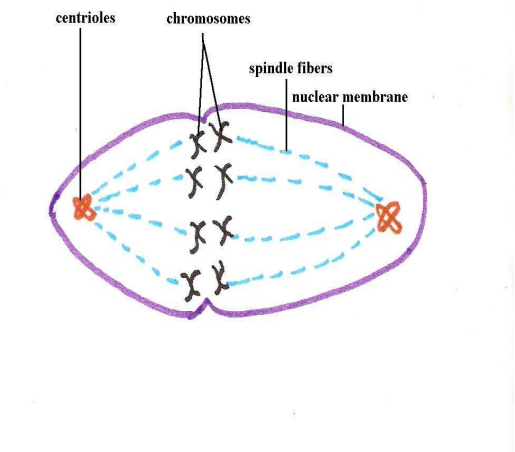
All the cells in the human body undergo cell division by mitosis. Gametogenisis is the only process in human beings in which cell division occurs by meiosis. After mitotic division two daughter cells are formed. Each one of these daughter cells inherit one copy of each chromosome of the mother cell. To achieve this uniform distribution of chromosomes to two daughter cells, the mother cell must replicate its chromosomes exactly once in the synthetic phase and must separate the replicated chromosomes evenly at the end of the mitotic phase to the two daughter cells.Defects in the coordination of chromosome replication and chromosome segregation can have severe consequences leading to genetic instability and aneuploidy which eventually can lead to cancer [1-3].

To ensure faithful transmission of chromosomes during cell division, eukaryotic cells have cellular regulatory mechanisms termed as cell cycle checkpoints [4]. The checkpoints prevent or delay cell cycle progression if certain cellular processes or proteins are disrupted, to gain time to repair the damage before cell division occurs. When the damage is irreparable, the cell undergoes apoptosis through the triggering of specific biochemical pathways [5]. However, cancer cells often harbor defective cell cycle checkpoints allowing for uncontrolled cell proliferation even when cell division does not occur properly. Therefore, effective cancer treatment can be achieved by drugs that target cell cycle machinery [6]. In this approach microtubules seem to be the best target because, apart from being the key component in cytoskeleton they are also responsible for the formation of spindle fibers during cell division (microtubules join together and form spindle fibers) which are responsible for segregation of chromosomes [Fig-1]. Thus the chemical compounds which particularly target microtubules and inhibit the abnormal function of the mitotic spindle should prove to be the best cancer chemotherapeutic agent. But this is not so because of the serious side effects produced by microtubule targeting drugs and the ability of cancer cells to develop resistance towards these drugs if used for a long period of time. In this article we will discuss about the microtubules, the agents that target microtubules like colchicine, vinca alkaloids and taxanes and

we will also discuss about the various approaches that are currently under consideration as the way to overcome the side effects of these drugs.

MICROTUBULE STRUCTURE

Fig-1: Segregation of Chromosomes by microtubules



Microtubules are polymers of protein called as α and β-tubulin which exist in the form of dimers [7]. The tubulin dimers polymerize end to end in head to tail fashion to form protofilaments [8]. The protofilaments associate longitudinally to form a sheet, which then closes up to form a microtubule with a diameter of 25 nm. These microtubules unite to form spindle fibers which take part in segregation of chromosomes during cell division [Fig-1]

α -Tubulin and β-tubulin are about 50% identical to each other in their composition of amino acids and the polymerization of α/β- tubulin heterodimers results in heterogeneity between the two ends of the microtubule. The end which terminates with α-tubulin is considered as less dynamic end and is called as minus end because; the GTP on α-tubulin is unexchangeable. The end which terminates with β- tubulin is considered as more dynamic end and is called as plus ends [9] because; the GTP on β-tubulin is exchangeable. Within a cell, microtubules are anchored by their minus ends at the microtubule-organizing center, disposing their plus ends to the cell periphery [10-11]. Microtubules are a component of the cytoskeleton. This description is a bit misleading however, since it suggests microtubules as static structures. Instead, microtubules are intrinsically dynamic polymers that grow and shorten by the reversible non-covalent association and disassociation of α/β-tubulin heterodimers at their two ends [12]

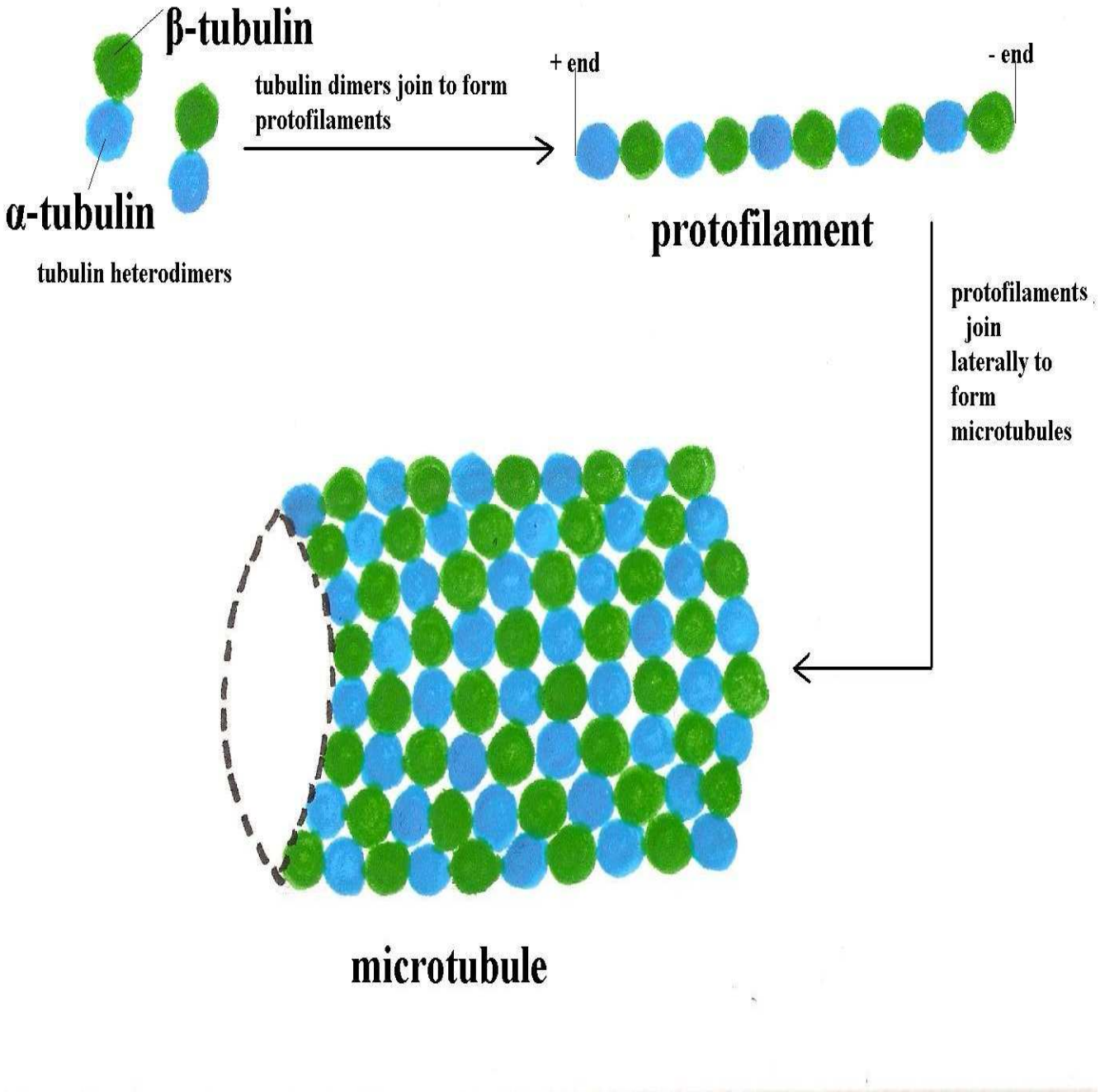


Fig-2 Formation of microtubule from tubulin heterodimers.

MICROTUBULE DYNAMICS

The α- tubulin and β-tubulin subunits each has a GTP binding site, referred to as the nonexchangeable site in α-tubulin and the exchangeable site in β-tubulin [13-14] . During the joining of α/β-tubulin heterodimer to the ends of microtubules, GTP in β-tubulin is hydrolyzed to GDP and the resulting GDP in β-tubulin is unable to exchange. When the microtubule depolymerizes, the α/β-tubulin heterodimers are released and the GDP in β-tubulin is now changes to GTP. In contrast, although α-tubulin also binds a GTP molecule, the GTP is bound at the nonexchangeable site and not able to be hydrolyzed to GDP during the addition of tubulin heterodimer to the ends of microtubules. The unique GTP binding and hydrolysis property on α-tubulin and β-tubulin gives microtubules two unusual dynamic properties, which are called as dynamic instability and treadmilling [15]. Dynamic instability refers to the ability of microtubule to switch between episodes of growth and shortening, and treadmilling describes the net growth of a microtubule at one end and net shortening at the other end. The dynamic properties of microtubules are crucial for many cellular functions, especially for proper spindle function during mitosis. In fact, spindle microtubules are 10-100 fold more dynamic than interphase microtubules to enable efficient capturing, alignment and segregation of chromosomes. As a result, suppression of microtubule dynamics impairs successful chromosome attachment and movement, and subsequently blocks cell cycle progression at mitosis through engaging the spindle checkpoint. The spindle checkpoint monitors both the proper attachment of chromosomes at their kinetochores to spindle microtubules, and the tension excerted across paired kinetochores by the kinetochore microtubules [16]. A growing body of evidence has shown that even a small alteration of microtubule dynamics can lead to improper attachment of chromosomes to spindle fibers and impair the kinetochore tension, which in turn signals the spindle checkpoint to prevent anaphase onset and chromosome segregation [17-18]. Due to this eventually mitosis stops and cell undergoes apoptosis [19-20].

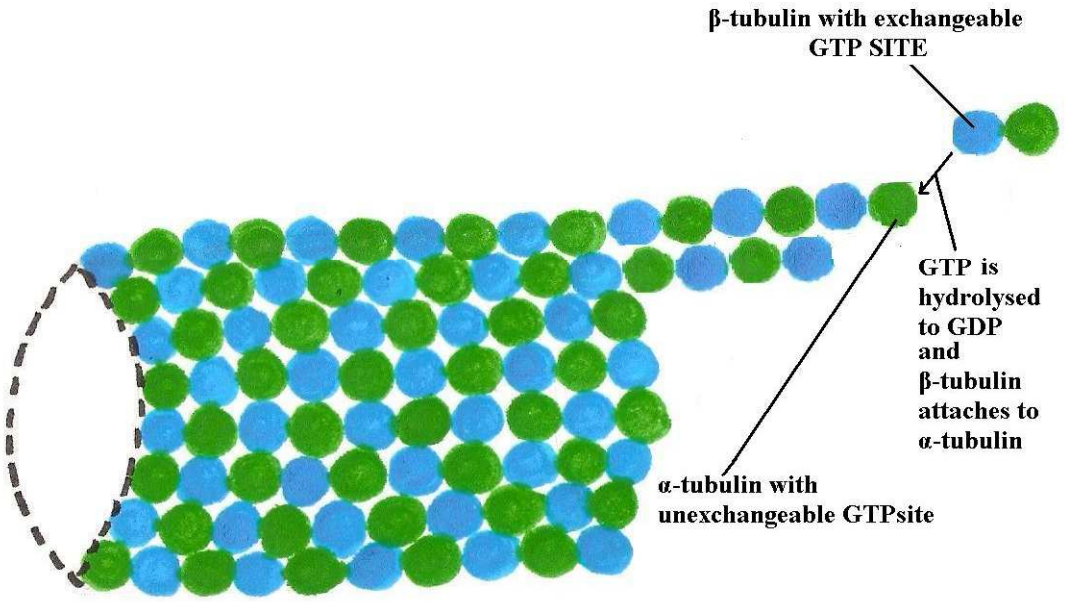


Fig-3: Elongation of microtubule.

MECHANISMS OF ACTION OF MICROTUBULE TARGETTING DRUGS

Chemical compounds targetting microtubules exert their inhibitory effects on cell proliferation primarily by blocking mitosis, which requires an exquisite control of microtubule dynamics. Microtubule-targeting drugs are therefore also frequently referred to as a group of anti-mitotic drugs, and their actions on microtubule stability and dynamic parameters differ from each other. At relatively high concentrations, these drugs either inhibit microtubule polymerization, destabilizing microtubules and decreasing microtubule polymer mass, or promote microtubule polymerization, stabilizing microtubules and increasing the polymer mass [21-22]. Microtubules targeting drugs like colchicine are known to cause

microtubule depolymerization which leads to vasoconstriction and the underlying mechanism responsible for this effect is still under investigation.

Based on these dramatic effects, microtubule targeting agents are divided into two traditional categories: microtubule-destabilizing agents such as the colchicine, vinca alkaloids and microtubule- stabilizing agents such as the taxanes (paclitaxel and docetaxel) . The anti-mitotic and anti-cancer activities of microtubule-targeting drugs have been thought to result from their actions on microtubule stability and polymer mass. However, at low but clinically relevant concentrations, both microtubule-stabilizing and -destabilizing drugs potently suppress microtubule dynamics without affecting microtubule polymer mass; however, they retain their ability to block mitotic progression and induce apoptosis [23] . Thus, it is reasonable to argue that the anti-mitotic and anti-cancer activities of microtubule-targeting agents may be largely due to their suppression of microtubule dynamics, instead of their effects on microtubule polymer mass, as previously assumed.

Currently there are three well established drug binding sites on β-tubulin, the vinca domain, the taxane site and the colchicine site[24]. The vinca domain is located adjacent to the exchangeable GTP binding site in β-tubulin at the plus end interface. The taxane site resides in a deep hydrophobic pocket at the lateral interface[25-26] between adjacent protofilaments, within the lumen of the microtubule[27-28]. Finally, the colchicine site is located at the intra-dimer interface between β-tubulin and α-tubulin . In addition to these three well characterized drug-binding sites, there is another binding site on β-tubulin that is occupied by laulimalide , a microtubule-stabilizing drug isolated from the marine sponge cacospongia mycofijiensis, however,the exact location of this binding site is still to be conformed[29-30].

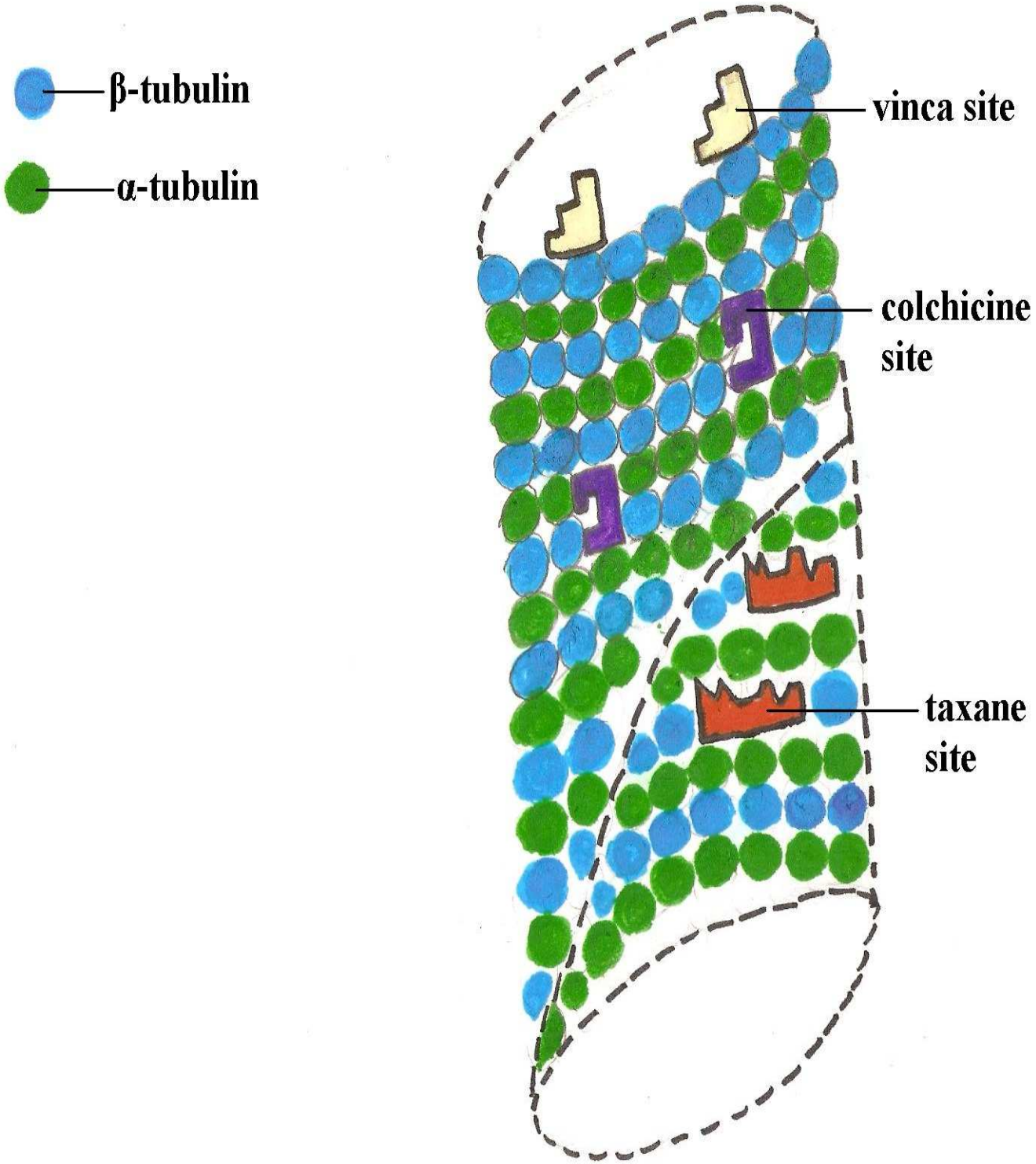


Fig-4 Binding sites on microtubules.

Agents which act on microtubules bind to any one of these sites on the microtubule and show their action on microtubules.

Table No.1 – Drugs binding to various sites on microtubules along with their clinical significance and their status of clinical development

Binding domain	**Drugs binding to the particular site**	**Clinical significance**	**Current status of the drugs and information about new combinations in clinical trials**	**References**
Vinca domain(natural derivatives)	Vinblastine (Velban)	Used in the treatment of Hodgkin's disease, testicular germ-cell cancer	Currently in clinical use and the clinical trials for 22 new combinations are in progress	[31-34]
	Vincristine(oncovin)	Used in the treatment of leukemia, lymphomas	Currently in clinical use and 108 new combinations are in clinical trials.	[35-36]
Semisynthetic and synthetic derivatives	Vinorelbine (Navelbine)	Used in the treatment of solid tumors,lymphomas,lung cancer	Currently in clinical use and 29 clinical trials are going on, on new combinations and on single drug	[37-39]

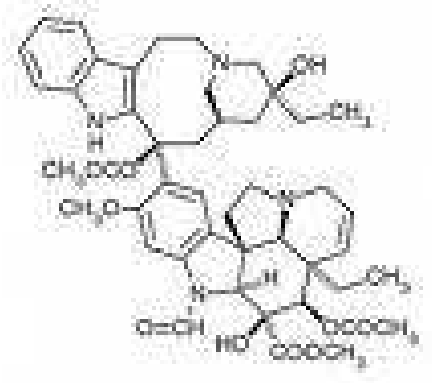
			which are in the phases ranging from Phase I–III	
	Vinflunine	Has shown significant activity against bladder, non-small-cell lung cancer, breast cancer	Clinical trials upto phase I-II have been successfully completed and phase III trails are in progress.	[40]
	Cryptophiycin 52	Is found to be effective against solid tumours	Clinical trials upto phase III have been successfully completed.	[41-42]
	Halichondrins	Still to be confirmed	In phase I clinical trials.	[43-45]
	Dolastatins	Is found to have ability to target tumor vasculature.	Has successfully completed Phase I and Phase II clinical trials.	[46]
	Hemiasterlins	Still to be conformed	In Phase I clinical trials.	[47-48]
Colchicine site(natural derivatives)	Colchicine	Non neoplastic diseases(gout,familial mediteraniaan fever)	Has failed in the clinical trials because of its toxic	[49-50]

			effects.	
Semisynthetic and synthetic derivatives	Combretastatins	Found to target tumor vasculature.	Has successfully passed in Phase I, II clinical trials	[51-52]
	2-Methoxyestradiol	Still under investigation.	Is in Phase I clinical trials.	[53-54]
	Methoxybenzene sulphonamide	Has shown some activity against solid tumours	Has passed in Phase I clinical trials and phase II clinical trials are in progress.	[55]
Taxane site(natural derivatives)	Paclitaxel (Taxol),	Is used in the treatment of ovarian, breast and lung tumours, Kaposi's sarcoma, is in clinical trials with numerous other tumours	Currently in clinical use and Phase I–III clinical trials are in progress in USA	[56-58]
Semisynthetic and synthetic derivatives	Docetaxel (Taxotere)	Used in the treatment of prostate, brain and lung tumours	8 clinical trials are in progress in the United States which are in the phases ranging from Phase I–III	[59-60]
	Epothilones	Is found to have activity against paclitaxel-resistant	Phase I–III clinical trials are in	[61-64]

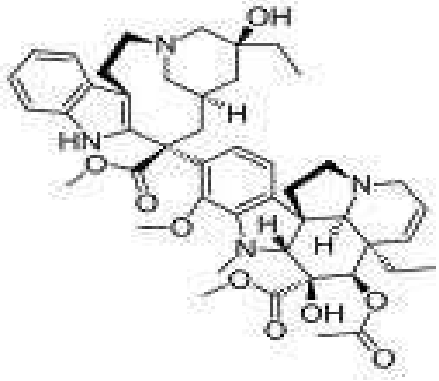
		tumours	progress.	
	Discodermolide	Still under investigation	Phase I clinical trials are in progress.	[65-69]
Other binding sites on microtubules	Estramustine	Is found to be effective against Prostate cancer.	Phases I–III, clinical trials in numerous combinations with taxanes, epothilones and vinca alkaloids are in progress.	[70-73]

SPECIFIC DRUG MECHANISMS

The vinca alkaloids bind to both tubulin and microtubules, and their actions are highly dependent on the drug concentration. At relatively high concentrations, they cause microtubule depolymerization, dissolve spindle microtubules and arrest cells in mitosis, and at even higher concentrations (μM), they induce the aggregation of tubulin into paracrystalline arrays[74] . In contrast, at low concentrations, the vinca alkaloids suppress microtubule dynamics without depolymerizing spindle microtubules, but remain able to arrest mitosis and induce apoptosis [75-77]



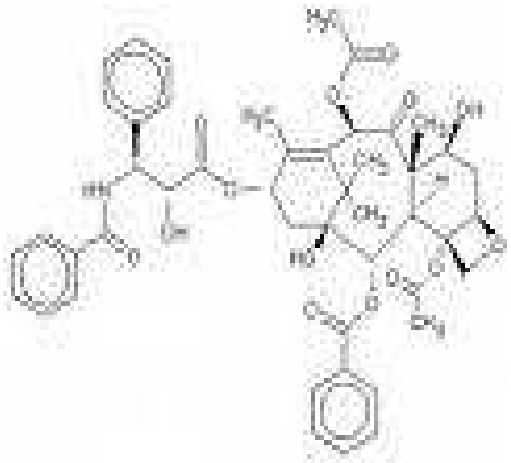
vincristine[78]



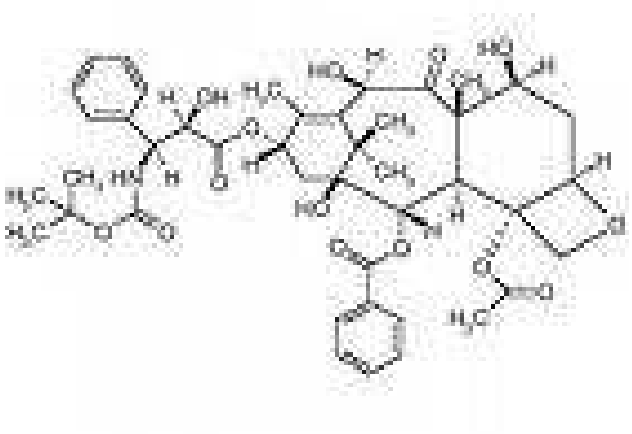
vinblastine[78]

Paclitaxel[79] enhances polymerization of tubulin, mechanism is opposite to that of vinca alkaloids. The microtubules are stabilized their depolymerization is prevented and.this stability results in inhibition of normal dynamic reorganization of the microtubule network that is essential for vital interphase and mitotic functions. Abnormal arrays or bundles of microtubules are produced through out the cell cycle. Cytotoxic action of paclitaxel emphasizes the importance of tubulin-microtubule dynamic equilibrium.

Docetaxel is a semi-synthetic derivative of paclitaxel and its mechanism of action is similar to that of paclitaxel.



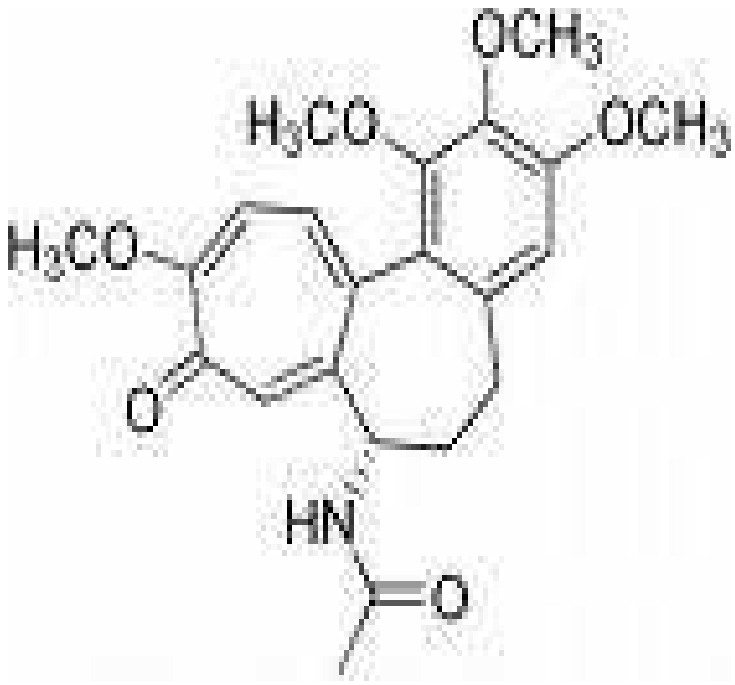
Paclitaxel[78]



Docetaxel[78]

Colchicine is one of the earliest microtubule-targeting agents identified, and its mechanism of action has been extensively investigated. In fact, tubulin was first purified based on its high-affinity binding with

colchicine and was referred to as a “colchicine-binding protein”[80]. Colchicine typically induces microtubule depolymerization at high concentrations, similar to the vinca alkaloids, and they suppress microtubule dynamics at low concentrations similar to both the vinca alkaloids and taxanes. Colchicine is probably the most powerful "radiomimetic" drug known ie., it reproduces the cellular changes induced in cells by x rays. Along with this colchicine also shows the highest affinity for microtubules, its affinity is so high that even at dilutions of 1 in 100,000,000[81] it can bring about the typical mitotic arrest at the metaphase stage and microscopically, areas of a tissue treated with colchicine will sometimes show more cells in arrested division than in the resting state[82]. Because of all these properties colchicine should prove to be the best drug for treatment of cancer but the clinical development of colchicine for cancer treatment has not been successful until now probably because of its high toxicity to normal tissues.



Colchicine[78]

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LIMITING FACTORS FOR THE EFFICIENT CLINICAL USE OF MICROTUBULE TARGETING DRUGS

Side effects:

Adverse side effects affect the applicability of microtubule targeting drugs in cancer therapy. Neurological and hematological side effects are the major ones and often dose limiting toxicities, but several other side effects also occur during the treatment with each individual drugs [88-84]. Peripheral neuropathy is the most frequently encountered neurotoxicity typified by the loss of deep tendon reflex at the ankle, numbness, and motor weakness. Cranial neuropathy may also occur after therapy with the vinca alkaloids and taxanes, resulting in jaw pain and vocal cord dysfunction. Autonomic neuropathy is another common symptom causing constipation, abdominal cramping, and urinary retention. Other central neurotoxicities include headache, dizziness, and mental depression. Neurotoxicity usually occurs after prolonged treatment with microtubule-targeting drugs, and is at least in part due to the inhibition of axonal microtubules, which are crucial for axonal transport in neurons.

Hematological toxicity is another major side effect caused by microtubule-targeting drugs, and is also frequently referred to as myelosuppression [83-84]. Severe neutropenia, in particular, occurs early after treatment. The cause of myelosuppression may result from the inhibition of the rapidly dividing hematopoietic cells. In addition to the neurological and hematological toxicities, microtubule targeting drugs may cause nausea, vomiting and diarrhea, but severe manifestations are uncommon.

Drug resistance:

Drug resistance, either intrinsic or acquired, is the major factor effecting the clinical applicability of microtubule targeting agents. The molecular basis underlying drug resistance is under intensive investigation. One of the most extensively studied mechanisms involves the over expression of drug efflux pumps, such as P-glycoprotein and multidrug resistance-associated protein 1 (MRP1), which are a family of ATP-dependent transporter proteins located in the cell membrane [85]. These drug efflux transporters

can efficiently pump anti-cancer drugs out of the cells, thereby lowering intracellular drug concentrations. This is probably the most efficient mechanism for cancer cells to achieve resistance to many structurally unrelated drugs. This phenomenon called as multidrug-resistance (MDR). Drugs that are affected by this mechanism include the microtubule-targeting drugs vinca alkaloids and taxanes, as well as a broad range of other classes of anti-cancer agents [86]. Alterations in tubulin/microtubules, represents another major mechanism underlying the resistance of cancer cells to microtubule-targeting drugs. These mechanisms include acquired tubulin mutations at the drug binding sites [87-90] , altered microtubule dynamics[91], altered expression of different tubulin isotypes [92], and changes in microtubule- regulatory proteins[93-94]. However we are not sure about the clinical significance of the drug efflux transporter-mediated mechanism and the microtubule-related mechanisms is still under investigation. In addition, besides the above mentioned mechanisms, cancer cells may employ the dysfunctions in apoptosis pathways, defects in cell cycle checkpoints, and altered drug metabolisms, together with many other unknown mechanisms to overcome the cytotoxic effects of microtubule-targeting drugs. Researchers are putting in a lot of efforts to overcome these limiting factors by different approaches like reducing the overall dose of drugs and by using various drug delivery systems.

APPROACHES TO OVERCOME THE LIMITING FACTORS

1) By using drug delivery systems for microtubule targeting:

Various drug delivery systems used in this approach include

Liposomes:

There are many potential barriers to the effective delivery of a drug in its active form to solid tumors. Most small-molecule chemotherapeutic agents have a large volume of distribution on i.v. administration [95]. The result of this is often a narrow therapeutic index due to a high level of toxicity in healthy tissues. Through encapsulation of drugs in a macromolecular carrier, such as a liposome, the volume of distribution is significantly reduced and the concentration of drug in the tumor is increased. This results in a decrease in

the amount and types of nonspecific toxicities and an increase in the amount of drug that can be effectively delivered to the tumor[96]. The liposome protects the drug from metabolism and inactivation in the plasma, and due to size limitations in the transport of large molecules or carriers across healthy endothelium, the drug accumulates to a reduced extent in healthy tissues[97] However, discontinuities in the endothelium of the tumor vasculature have been shown to result in an increased extravasation of large carriers and, in combination with an impaired lymphatics, an increased accumulation of liposomal drug at the tumor [99]. All of these factors have contributed to the increased therapeutic index observed with liposomal formulations of some chemotherapeutic agents [99].

Table No- 2: Various drugs used in microsomal form for microtubule targeting.

Name of the drug administered in liposomal form	**Composition of liposomes**	**Advantages over drug in free form**	**references**
Liposomal vincristine	Egg phosphatidylcholine (EPC)/cholesterol (55/45, mol/mol) and distearoylphosphatidylcholine (DSPC)/cholesterol (55/45, mol/mol)	On comparision with free drug it shows decreased toxicity and increased antitumor efficacy.	[100]
Liposomal colchicine	Made up of phospholipids	It is still under study and is expected to have lesser toxicity over colchicine in free form and thus is	[101]

		expected to bring colchicine into clinical use.	
Liposomal paclitaxel	Poly ethylene glycolated lipids	Has lesser side effects when compared to currently used formulation ie…taxol.	[102-104]

Nanocarriers:

There has been a great increase in the use of nano particles as carriers for drug molecules because these are easy to produce and can be stored at normal storage conditions. Nowadays carbon nanotubes are mostly used as carriers. Along with these many other substances like poly lactic-co-glycolic acid, vitamin E TGPS(tocopheryl polyethylene glycol succinate), niosomes(Nonionic surfactant vesicles which result from the organized assembly of sufficiently insoluble surfactants in aqueous media) and Nano liposomes(These are the liposomes whose radius is in nano meters) are also used. The main advantage of the nano carriers is there is a exhaustive list of substances which can be used as nano carriers.

Table No-3: Various microtubule targeting drugs administered using nano carriers.

Name of the drug	**Nano carrier used**	**Status of clinical development**	**references**
paclitaxel	Poly lactic-co-glycolic acid	Still in the early stages of development and	[105]

		clinical trails are not yet started	
colchicine	vitamin B_{12}	Still in the early stages of development and clinical trails are not yet started.	[106]
Vincristine, vinblastine	Nanoliposomes	Still in the early stages of development and Phases I clinical trails are going on	[107]

2) By using synergistic combinations of drugs:

When two or more drugs which act synergistically are given in a combination the over all dose of the drug can be reduced due to combined action of drugs and thus the adverse effects can be reduced

Table-4: Examples of synergistic combinations of drugs.

Combinations of drugs used	Advantages over single drug	references
Estramustine with vinblastine and Estramustine with paclitaxel	Antiproliferative ability of estramustine is additive with that of vinblastine and paclitaxel thus low dose is enough	[108-110]
Vinorelbine plus paclitaxel and Vinorelbine plus docetaxel	Superior to either drug alone, because binding is on both vinca	[111-112]

	site and taxane site	
paclitaxel and discodermolide	Higher microtubule suppression on comparision with either drug alone	[113]
docetaxel and the colchicine analogue CI-980	Superior to either drug alone, because binding is on both taxane site and colchicine site	[114]

CONCLUSIONS AND FUTURE OF MICROTUBULE TARGETING DRUGS

It is reasonable to argue that microtubules represent the best target to date for cancer chemotherapy and will remain a promising target for new chemotherapeutic agents. The drugs like vinca alkaloids, taxanes and colchicine are the active principles with highest affinity for the microtubules on comparision with any other drugs and these drugs act as potent antimitotics. But these drugs could not be used for complete cure of cancer because, their potential to cure cancer could not be exploited fully due to their side effects and development of drug resistance. Future challenges in the use of microtubule-targeting agents lie in increasing the understanding of their basic mechanisms and improving their clinical effectiveness. For example, microtubule-targeting drugs could be used in combination therapy by using synergistic combination of drugs at much lower doses than the doses which are presently considered as biologically effective doses (that suppress microtubule dynamics) rather than at their maximum tolerated doses. Furthermore, relatively weak microtubule-targeting drugs that suppress dynamics (for example, griseofulvin, coumarins and benomyl) could be used as adjuvants in chemotherapy to attain efficacy with decreased toxicity. The maintenance of low concentrations of microtubule-targeting drugs in tumour tissue for long durations could be more effective in tumour-cell killing than the rapidly rising and falling drug concentrations due to the administration of maximum tolerated doses. Finally, because of the involvement

of microtubules in so many different cellular processes, they could be combined very effectively with 'molecularly targeted' drugs. With regard to improving the basic knowledge of these drugs, we need to understand the relationship between drug-induced mitotic block and cell death, as well as the interactions of the microtubule-targeted drugs with the centrosome or mitoticspindle pole, where other forms of tubulin(other than α and β-tubulin) are present. In addition, the reasons for development of resistance to microtubule targeted drugs, such as tubulin mutations have to be studied thoroughly. If microtubule-targeting drugs that can overcome Multi drug resistance and neuropathy are developed, it will be a great clinical achievement. Understanding the reasons for specificity of action like why the taxanes work well in ovarian and breast cancer, whereas the vinca alkaloids often work well in blood cancers can lead to development of more efficient drugs. Finally if the microtubule targeting drugs are studied properly and if we can find ways to exploit their properties fully then the cancer treatment can be revolutionized and the complete cure of cancer will be possible

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